ORIGINAL ARTICLE VIROLOGY

Adenovirus infection and disease in paediatric haematopoietic stem cell transplant patients: clues for antiviral pre-emptive treatment

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Abstract

Human adenovirus (HAdV) infections constitute a major cause of morbidity in paediatric haematopoietic stem cell transplant (HSCT) patients. New antiviral treatments offer promising perspectives. However, it remains challenging to identify patients at risk for disseminated infection, and who should receive early antiviral intervention. We conducted a longitudinal study of allogeneic HSCT recipients, including weekly HAdV monitoring, to determine the risks factors associated with HAdV infection and dissemination, and to assess whether HAdV loads in stools may be used as surrogate markers for HAdV dissemination. Between September 2010 and December 2011, out of 72 patients, the cumulative incidence rates at day 100 of HAdV digestive infection, systemic infection and related disease were 35.9%, 24.0%, and 18.3%, respectively. In multivariate analysis, the risk factors for HAdV digestive and systemic infection were cord blood and *in vitro* T-cell depletion. Graft-versus-host disease (GVHD) grade >2 was also associated with systemic infection. In patients with HAdV digestive shedding, GVHD grade >2 and HAdV load in stools were the only risk factors for systemic infection. The median peak levels of HAdV in stool were 7.9 and 4.0 $\log_{10}$ copies/mL, respectively, in patients with HAdV systemic infection and in those without. HAdV monitoring in stools of paediatric HSCT recipients receiving cord blood or *in vitro* T-cell depleted transplants helps to predict patients at risk for HAdV systemic infection. Our results provide a rationale for randomized controlled trials to evaluate the benefit of anti-HAdV pre-emptive treatments based on HAdV DNA levels in stools.

Keywords: Adenovirus, adenovirus disease, adenovirus infection, antiviral treatment, dissemination, haematopoietic stem cell transplant, plasma, stool

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Introduction

Viral diseases constitute a major cause of morbidity and mortality after haematopoietic stem cell transplantation. Whereas reactivations of *Herpesviridae* family viruses are usually well controlled through effective monitoring and antiviral treatments, human adenovirus (HAdV) infections remain associated with high morbidity in paediatric haematopoietic stem cell transplant (HSCT) patients [1].

Although HAdVs do not produce lifelong infections, they may persist and lead to endogenous reactivations, especially from the digestive tract in immunocompromised individuals [2–4]. Paediatric HSCT recipients are more susceptible to adenovirus infection than are adults. The incidence of HAdV

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TABLE 1. Patient characteristics, transplantation modalities, and outcomes

Parameters	Value	N	Median (IQR) or %
Age at SCT (years)		72	6.5 (3.8–11.6)
Gender	Male	42	58.3
	Female	30	41.7
Diagnosis	AML	9	12.5
	ALL	21	29.2
	JMML	9	12.5
	Lymphoma	5	6.9
	Haemoglobinopathy	11	15.3
	Others	17	23.6
TBI	Yes	22	35.5
Allograft in CR1	Yes	45	62.5
Stem cell source	Peripheral blood	5	7.0
	Bone marrow	53	73.6
	Cord blood	14	19.4
Donor type	Geno-identical	24	33.3
	Haplo-identical	5	6.9
	≥9/10 HLA-unrelated	29	40.3
	≥4/6 cord blood	14	19.4
Acute GVHD	No	31	43.1
	Grade ≤2	10	13.9
	Grade >2	25	34.7
	Prior death	6	8.3
HAdV digestive infection	Yes	28	38.9
HAdV systemic infection	Yes	18	25.0
HAdV probable disease	Yes	13	18.1
HAdV disease symptoms	Fever	10	76.9
	Diarrhoea	10	76.9
	Respiratory disease	7	53.9
	Hepatitis	11	84.6
	Encephalitis	3	23.1
	Multivisceral failure	1	7.7
	Pancreatitis	1	7.7
	Haemorrhagic cystitis	2	15.4
Death	M3	7	9.7
	M12	13	16.7

ALL, acute lymphoid leukaemia; AML, acute myeloid leukaemia; GVHD, graft-versus-host disease; HAdV, human adenovirus; HLA, Human Leucocyte Antigen; IQR, interquartile range; JMML, juvenile myelomonocytic leukaemia; M3, 3 months after graft; M12, 12 months after graft; SCT, stem cell transplantation; TBI, total body irradiation.

viraemia has been reported to be 6–28% in children, and only up to 6% in adults [3–7].

Treatment options are limited [8]. Cidofovir and ribavirin are antiviral drugs that are effective *in vitro*, although their use has not been validated with randomized trials [4,8,9]. Some new strategies under clinical investigation have been raising new hopes. First, CMX001 (brincidofovir), a lipophilic conjugate of cidofovir, providing a larger tissue distribution and a higher intracellular concentration than cidofovir, has shown encouraging results in a few case reports [10,11]. Second, the infusion of anti-HAdV T-cells expanded from the donor or from a third party has been reported to be an effective strategy, provided that the treatment can be initiated quickly to enable control of viral replication [12,13]. Because early interventions are key to antiviral efficacy [8,13], there is a need, first, to identify patients with a high risk of HAdV disseminated infection, and second to define when to initiate pre-emptive or curative treatment.

In this study, we assessed the risk factors associated with HAdV digestive shedding, blood infection and HAdV disease in a single paediatric HSCT centre, and determined whether HAdV loads in stools and plasma may be used as surrogate markers for HAdV dissemination. In agreement with previous reports [3,14], the risk factors that we have identified will make it possible to target patients requiring a narrow follow-up, and the HAdV load levels in stools that we have defined may be used to trigger therapeutic intervention.

Patients and methods

Patients

Between September 2010 and December 2011, 72 children (30 females and 42 males) who underwent allogeneic stem cell transplantation at Robert Debré Hospital (Paris, France) were prospectively followed for up to 12 months. Patient characteristics and modalities of transplantation are summarized in Table 1. Details of medical history and clinical follow-up are given in Doc. S1.

Patients with probable HAdV disease were treated with cidofovir. If renal function was normal, cidofovir was administered at 5 mg/kg once weekly with probenecid. If renal function was impaired (creatinine level twice the normal level), cidofovir was administered at 1 mg/kg three times weekly with probenecid. Ribavirin or brincidofovir was given to patients not responding to cidofovir. Brincidofovir was used only when it was available for compassionate use. When possible, immunosuppressive therapy was tapered. Two patients received specific anti-HAdV cytotoxic lymphocytes from either a haploidentical related donor or a third-party donor. Cidofovir was given to some patients who had persistent high HAdV DNA levels in stools and were considered to be at high risk for disseminated infection.

Ethics statement

The study was carried out in accordance with the Declaration of Helsinki. This study was a non-interventional study with no additional procedures. Biological material and clinical data were obtained only for standard viral diagnostic according to physicians' prescriptions. Data analyses were carried out with an anonymous database. According to the French Health Public Law (CSP Art L 1121-1.1), such a protocol is exempt from informed consent application. The two parents or guardians of these paediatric patients gave written informed consent to all aspects of the transplantation procedure and to the use of medical records for research.

Virus monitoring

Whole blood samples were collected weekly and tested for cytomegalovirus (CMV) and Epstein–Barr virus by quantitative PCR. HAdV infections were monitored on a weekly basis by quantitative PCR in plasma and stool samples until discharge or

up to day 100 after transplantation. After discharge, HAdV monitoring was continued for up to 12 months, including mainly plasma samples.

Definitions

Local digestive infections were defined as positive HAdV PCR in stools, systemic infections as positive HAdV PCR in plasma, and probable HAdV disease as a systemic infection in association with compatible symptoms, without other identifiable causes, including fever, respiratory diseases, haemorrhagic cystitis, hepatitis, pancreatitis, meningoencephalitis, multi-organ failure, and diarrhoea with any of the previous symptoms. HAdV-related enteritis without any other symptoms related to HAdV infection was not included as probable HAdV disease.

Adenovirus quantification in stool and plasma specimens

The extraction procedures for stool specimens and plasma are given in Doc. S1. HAdV DNA was quantified with the Adenovirus Rgene kit (Argene, Varhilles, France). The results were expressed as copies/mL of the stool phosphate-buffered saline suspension. The threshold for quantification was 200 copies/mL.

Adenovirus typing

HAdV species identification was performed with real-time PCR assays (details are given in Doc. S1) [15]. HAdV type was determined by sequencing of hypervariable region 7 of the hexon gene and fibre [16,17].

Statistical analysis

Summary statistics were computed, either medians with interquartile range (IQRs), or percentages. Cumulative incidence curves of HAdV infection and disease were computed in a competing-risks framework, wherein death prior to the event of interest was considered as a competing event. Cause-specific Cox models were used to assess the predictive set of covariates associated with each outcome, with multivariable models incorporating covariates that were selected by the univariate analyses at the 10% level; viral loads on $\log_{10}$ scales were introduced as time-dependent covariates. Cubic smoothing spline functions in generalized additive models allowed us to plot the hazard of event along the covariate scale, testing for non-linearity [18]. Point estimates of cause-specific hazard ratio (HR) were reported with 95% CIs.

Statistical analyses were performed with SAS V9.3 (SAS, Cary, NC, USA) and R 2.13.0 (http://www.R-project.org/) packages. All tests were two-sided, with p-values of ≤ 0.05 denoting statistical significance.

Results

Adenovirus digestive infections, systemic infections, and disease

The cumulative incidence rates of HAdV digestive infection, systemic infection and related disease at day 100 following transplantation were 35.9% (95% CI 34.7–37.1%), 24.0% (95% CI 23.0–25.0%), and 18.3% (95% CI 17.4–19.2%), respectively (Fig. 1).

Adenovirus detection in stools

Adenovirus was detected in stools in 28 patients (39%). Of the 28 patients with HAdV intestinal shedding, 21 (75%) had diarrhoea, including 13 with a diagnosis of digestive graft-versus-host disease (GVHD). Viral loads at the first detection ranged from 2.4 $\log_{10}$ to 9.99 $\log_{10}$ copies/mL. The maximal HAdV viral load in stools was significantly higher in patients with diarrhoea (median 5.5 $\log_{10}$ copies/mL; IQR 3.5–8.0) than in those without diarrhoea (median 2.5 $\log_{10}$ copies/mL; IQR 2.4–3.3) (p 0.05). The median duration of HAdV digestive infection was 27 days (IQR 9.5–99). There was a strong predominance of species C (57%) (Table 2).

Adenovirus systemic infection

HAdV was detected in plasma in 18 patients (25%). One patient had remained negative for HAdV in stools before the positive

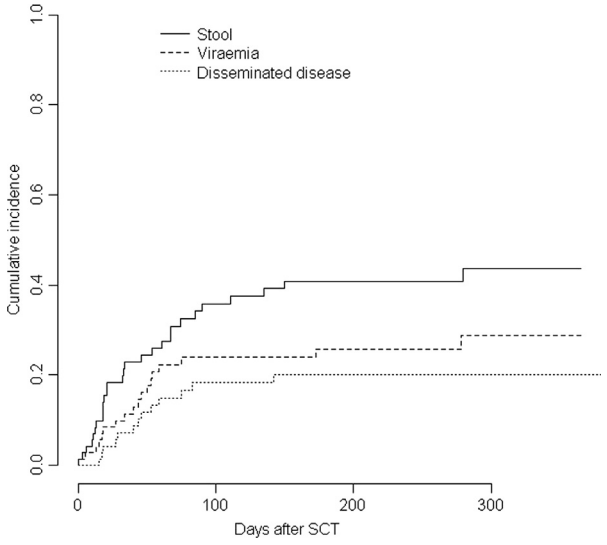

FIG. 1. Cumulative incidence of human adenovirus (HAdV) digestive infection, systemic infection and probable disease after human stem cell transplantation (SCT). The 100-day cumulative incidence rates of HAdV digestive infection (solid line), systemic infection (dashed line) and probable disease (dotted line) were 35.9%, 24.4%, and 18.3%, respectively.

TABLE 2. Viral characteristics of human adenovirus (HAdV) digestive infections, systemic infections, and related diseases

	HAdV digestive infection	HAdV systemic infection	Probable HAdV disease
Number of cases (*n*)	28	18	13
Minimal viral load[a]	3.3 (2.7–5.1)	0 (0–0)	0 (0–0)
First viral load[a]	4.5 (3.4–7.0)	2.7 (2.6–3.1)	2.8 (2.567–3.074)
Maximal viral load[a]	7.0 (4.0–8.3)	4.4 (3.1–5.4)	4.7 (3.16–5.475)
Species, *n* (%)			
A	5[b] (18)	3 (17)	2 (15)
B	2 (7)	2 (11)	2 (15)
C	16 (57)	10 (56)	7 (54)
D	1 (3.5)	1 (5.3)	1 (8)
F	1 (3.5)	1 (5.3)	0 (0)
Undetected[c]	3 (11)	1 (5.3)	1 (8)
Type, *n*			
A-12	1	1	0
A-31	4[b]	2	2
B-3	1	0	0
B-11	1	1	1
C-1	3	2	2
C-2	9	6	4
C-5	3	1	1
B undetected[c]	0	1	1
C undeteted[c]	1	1	0
D undetected[c]	1	1	1
F-41	1	1	0

[a]Median (interquartile range). For HAdV digestive infection, HAdV loads indicated are those determined in stools, and for HAdV systemic infection and probable HAdV disease, HAdV loads indicated are those determined in plasma. HAdV loads are expressed as $\log_{10}$ copies/mL.
[b]A co-infection with HAdV C2 occurred during the follow-up of a patient infected with HAdV A31.
[c]HAdV loads were too low for HAdV typing.

detection in plasma. HAdV viraemia was detected within 6 months for all patients but one, who, 10 months after transplantation, experienced an enteritis episode related to HAdV type 41. The plasma HAdV load at the first detection ranged from 2.3 $\log_{10}$ to 4.5 $\log_{10}$ copies/mL. The maximal viral load ranged from 2.42 $\log_{10}$ to 9.75 $\log_{10}$ copies/mL. The median duration of HAdV systemic infection was 24 days (IQR 14–63). Species C was the most frequent species associated with viraemia (56%). There was perfect concordance between the types detected in stools and those detected in plasma. Nosocomial transmission from a patient infected with an HAdV species C type 2 to two other patients was suspected (not shown). If these two cases had been prevented, the prevalence of HAdV systemic infection in HSCT children would have decreased from 24% (95% CI 15.5–36.6%) to 22% (95% CI 13.3–33.6%).

Adenovirus disease

Thirteen patients (72%) with HAdV viraemia experienced HAdV disease, including one who had stool samples negative for HAdV before the detection in plasma. All occurred before day 100 after transplantation. The main clinical signs are shown

TABLE 3. Univariate analyses of predictive factors for human adenovirus (HAdV) digestive infection, systemic infection, and disease (all variables associated in univariate analyses at the 10% level (indicated by *) were incorporated in the multivariable models

	HAdV digestive infection	HAdV systemic infection	Probable HAdV disease
	In all patients	In all patients	In all patients
	28 events/72 patients	18 events/72 patients	13 events/72 patients
Age <6 years	2.4 (1.12–5.14)*	2.3 (0.89–5.94)*	1.6 (0.54–4.83)
Allograft in CR1	2.0 (0.88–4.55)	2.7 (0.88–8.15)*	1.6 (0.48–5.06)
Non-geno-identity	3.0 (1.15–7.99)*	5.2 (1.2–22.8)*	3.3 (0.74–15.0)
Cord blood	4.9 (2.17–11.0)*	3.0 (1.11–8.04)*	2.6 (0.80–8.46)
Corticosteroids[a]	2.9 (1.08–7.55)*	2.8 (0.93–8.59)*	1.7 (0.37–7.57)
Fludarabine[b]	1.8 (0.84–3.78)	1.9 (0.74–4.78)	1.0 (0.31–3.33)
Endoxan[b]	0.6 (0.28–1.28)	0.4 (0.13–0.99)*	0.6 (0.20–1.84)
GVHD grade >2	1.2 (0.55–2.58)	2.6 (1.03–6.56)*	2.8 (0.95–8.43)*
In vitro T-cell depletion	3.7 (1.22–11.0)*	3.6 (1.03–12.8)*	3.0 (0.66–13.6)
ATG	0.8 (0.36–1.67)	0.7 (0.33–2.18)	1.2 (0.35–3.73)
Previous positive HAdV stool	—	2.4 (0.84–7.10)	1.9 (0.56–6.15)
Previous HAdV systemic infection	—	—	4.4 (1.20–16.4)*
		In patients with HAdV digestive infection 15 events/28 patients	In patients with HAdV systemic infection 13 events/18 patients
Age <6 years	—	1.3 (0.46–3.90)	0.7 (0.25–2.21)
Allograft in CR1	—	1.9 (0.52–6.64)	0.5 (0.16–1.78)
Non-10/10 HLA pheno-identity	—	3.9 (0.51–29.6)	0.5 (0.11–2.35)
Cord blood	—	1.3 (0.47–3.72)	0.8 (0.24–2.59)
Corticosteroids[a]	—	1.6 (0.52–5.10)	0.4 (0.12–2.47)
Fludarabine[b]	—	1.3 (0.46–3.48)	0.5 (0.16–1.70)
Endoxan[b]	—	0.3 (0.09–1.18)*	1.9 (0.60–5.74)
GVHD grade >2	—	5.0 (1.63–15.6)*	1.2 (0.40–3.51)
In vitro T-cell depletion	—	1.8 (0.49–6.28)	0.9 (0.20–4.08)
ATG	—	0.7 (0.24–1.84)	1.4 (0.42–4.45)
Stool HAdV load	—	1.4 (1.09–1.79)*	0.9 (0.75–1.03)
Plasma HAdV load	—	—	1.1 (0.84–1.46)

ATG, anti-thymoglobulin; CR1, First Complete Remission; GVHD, graft-versus-host disease; HLA.
Hazard ratio (Confidence interval 95%).
[a]In preventing GVHD.
[b]As conditioning regimen.

in Table 1. The maximal HAdV viral load in plasma was not significantly higher in patients with HAdV disease (median 4.7 log_{10} copies/mL; IQR 3.16–5.48) than in those without (median 3.5 log_{10} copies/mL; IQR 3.077–3.813) (p 0.44). However, an HAdV plasma load of >4 log_{10} copies/mL was observed in nine patients with HAdV disease and in one without.

Risk factors for digestive infection, systemic infection, and HAdV disease

Tables 3 and 4 show the predictive factors for HAdV positivity in stools, HAdV systemic infection, and HAdV-related disease.

This allowed selection at the 10% level for digestive infection, young age, non-geno-identity, cord blood transplantation, corticosteroid use for the prophylactic treatment of GVHD, and *in vitro* T-cell depletion. Of those variables, only cord blood transplantation and *in vitro* T-cell depletion were significantly associated with outcome, based on a multivariable cause-specific Cox model (Table 4).

Among the eight selected predictive factors for systemic infection in univariate analysis (Table 3), acute GVHD, cord blood transplantation and *in vitro* T-cell depletion were the three retained in the multivariable model (Table 4). Introducing the past occurrence of HAdV intestinal shedding as a time-dependent covariate into the multivariable model did not modify this finding.

The occurrence of GVHD grade >2 and previous HAdV systemic infection were both associated with HAdV disease at the 10% level in univariate analyses (Table 3). Only previous HAdV systemic infection remained predictive for HAdV disease in the multivariable model (Table 4).

It is of note that the median total lymphocyte count did not differ between patients with and without HAdV digestive infection (0.9×10^9/L vs. 1.2×10^9/L; p 0.55), HAdV viraemia (1.2×10^9/L vs. 0.8×10^9/L, p 0.48), and HAdV-related disease (1.3×10^9/L vs. 0.9×10^9/L; p 0.22) (Table S1).

TABLE 4. Multivariable analysis of predictive factors for human adenovirus (HAdV) digestive infection, systemic infection, and disease: results of the final model stepwise selection

Multivariable models	HR (95% CI)	p
HAdV digestive infection, 28 events/72 patients		
Cord blood	6.0 (2.57–14.0)	<0.0001
In vitro T-cell depletion	5.8 (1.82–18.3)	0.0029
HAdV systemic infection, 18 events/72 patients		
Cord blood	3.8 (1.33–10.9)	0.013
GVHD grade >2	2.8 (1.10–7.20)	0.030
In vitro T-cell depletion	5.3 (1.38–20.3)	0.015
HAdV systemic infection after HAdV digestive infection, 15 events/28 patients		
GVHD grade >2	4.5 (1.28–15.6)	0.019
Stool HAdV load	1.4 (1.04–1.74)	0.023
Probale HAdV disease, 13 events/72 patients		
Previous HAdV systemic infection	4.4 (1.20–16.4)	0.026

GVHD; graft-versus-host disease; HR, hazard ratio.

In this population, patients showed other viral reactivations. Twenty-five patients showed reactivation of CMV in blood, 43 showed an Epstein–Barr virus infection, nine showed a BK virus infection, and three showed a human herpesvirus 6 infection. Only the occurrence of CMV systemic infection was associated with that of HAdV (11 (61%) in patients with HAdV systemic infection vs. 14 (26%) in patients without; p 0.01).

Association of HAdV load in stools and blood with viraemia and disease

When the univariate analyses were restricted to the 28 patients with intestinal shedding of HAdV, the occurrence of systemic infection was increased in cases of acute GVHD grade >2, as well as with the maximal viral load detected in stools, whereas it was decreased by the use of endoxan in the conditioning regimen (Table 3). The median peak levels of HAdV in stools were 7.89 log_{10} copies/mL (IQR 6.96–9.31) and 4.03 log_{10} copies/mL (IQR 3.78–5.54), respectively, in the 15 patients who experienced HAdV systemic infection and in those 13 who did not (p 0.003). Fig. 2 shows how the maximal HAdV load in stools may predict infection, demonstrating that the higher the viral load, the higher the risk of infection. In a multivariable Cox model, only the viral load in stools (p 0.023) and the occurrence of GVHD grade >2 (p 0.02) were associated with the occurrence of systemic infection in patients with intestinal

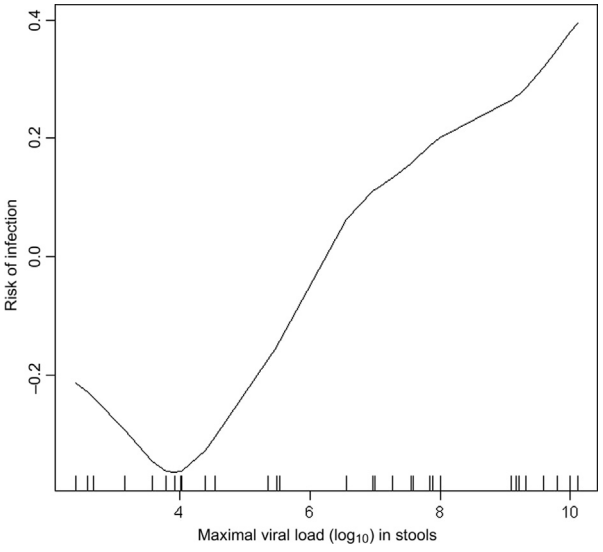

FIG. 2. Estimation of the risk of human adenovirus (HAdV) viraemia as a function of the HAdV viral load in stools, by the use of smoothing splines; estimation of how the maximal value of HAdV viral load in stools modifies the risk of HAdV viraemia from generalized additive models. For each of the 28 patients with HAdV digestive infection, the value of the maximal HAdV load detected in stools is indicated on the *x*-axis with a vertical dash, and expressed in log_{10} copies/mL.

shedding (Table 4). Fig. 3 shows the receiver operating characteristic curve for all possible cut-offs, with an estimated area under the curve of 0.83. This illustrates the discriminative capacity of such a measure to distinguish between the presence and absence of systemic infection. At a threshold of 5 $\log_{10}$ copies/mL HAdV in stools, the sensitivity and specificity for detecting a systemic infection in patients with HAdV intestinal shedding were 93.3% and 61.5%, respectively.

When the analysis was restricted to the 18 patients with HAdV viraemia, no factors allowed prediction of the occurrence of disseminated disease (Table 3).

Antiviral treatment

All patients with HAdV-related disease received antiviral treatment. Eleven patients received one to eight injections of cidofovir. Following cidofovir treatment, two patients also received ribavirin, four received brincidofovir, and one received ribavirin and then brincidofovir. Two patients also received anti-HAdV cytotoxic lymphocytes. Of these 11 patients, six died from HAdV-related disease. Two patients received only CMX001. Both died, including one because of an HAdV-related disease.

Four patients received two to five injections of cidofovir because they had HAdV digestive infection with high HAdV DNA levels, and were considered to be at high risk for disseminated infection. One patient with persistent high HAdV DNA levels in stools despite cidofovir treatment received brincidofovir. Each of these four patients eventually cleared the HAdV infections, and none had HAdV viraemia.

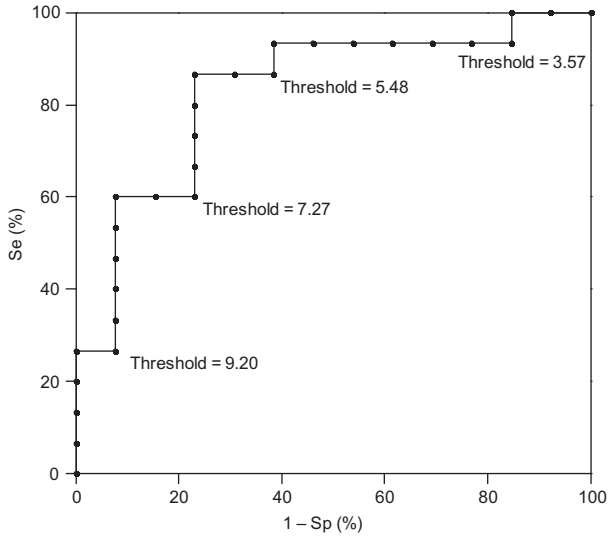

FIG. 3. Receiver operating characteristic curves showing sensitivity (Se) vs. 1 – specificity (Sp) for the prediction of human adenovirus (HAdV) disease with different positive cut-off values for HAdV load in stools.

Adenovirus-related and transplant-related mortality

Of the 72 patients studied, 13 died (18%) within 1 year after transplantation. The deaths were attributed to HAdV in seven, relapse in three, *Pseudomonas aeruginosa* septicaemia in two, and respiratory failure in one. Of the 13 patients with HAdV-related disease, eight died, including seven whose deaths were attributed to HAdV, and one whose death was attributed to respiratory failure without HAdV respiratory infection. CMV infection (HR 8.1, 95% CI 1.03–63.0) and the use of fludarabine (HR 5.0, 95% CI 1.49–16.6) and melphalan (HR 4.3, 95% CI 1.36–13.6) in the conditioning regimen were identified as baseline prognostic factors in univariate analyses of overall survival. Additionally, the risk of death in patients who experienced HAdV systemic infection was significantly increased as compared with patients who remained negative for HAdV in plasma (HR 9.5, 95% CI 2.77–32.5; p 0.0003). Otherwise, the risk of death was decreased in cases of anti-thymoglobulin use (HR 0.3, 95% CI 0.09–0.93; p 0.037) but increased in cases of *in vitro* T-cell depletion (HR 8.9, 95% CI 2.57–30.7; p 0.0006). Besides HAdV infection (HR 11.4, 95% CI 2.53–51.7; p 0.0015), multivariable analysis retained only the use of fludarabine (HR 8.30, 95% CI 2.07–33.2; p 0.0028) and CMV infection (HR 13.5, 95% CI 1.56–116.4; p 0.018) as adding prognostic information to each other.

Discussion

In our series, during the first 100 days following transplantation, the incidence rates of HAdV digestive infection, systemic infection and HAdV-related disease were 35.9%, 24.0%, and 18.3%, respectively. Our data agree with previous studies conducted in paediatric HSCT patients, and confirm that HAdV infections are common in paediatric HSCT patients [3,4,7,14]. Another recent study found an even higher frequency of HAdV systemic infection in paediatric HSCT patients (50.4%), despite a similar rate of HAdV disease (11.3%) [19].

We have reported the following main risk factors: cord blood and *in vitro* T-cell depletion for HAdV digestive infection; cord blood, GVHD grade >2 and *in vitro* T-cell depletion for HAdV systemic infection; and HAdV viraemia for probable HAdV disease. Our results confirm the association between the occurrence of HAdV replication and cord blood transplantation, severe GVHD, and *in vitro* T-cell depletion [3,4]. Therefore, patients receiving cord blood transplants or *in vitro* T-cell-depleted transplants, and those presenting with severe GVHD, should benefit from weekly molecular detection of HAdV within the first 100 days following transplantation.

In contrast to some other studies, our multivariable analysis did not show unrelated donors, or lymphopenia, as risk factors

for HAdV infection. Also, *in vivo* T-cell depletion with anti-thymoglobulin was not predictive of HAdV infection or disease. Several studies have reported discordant results regarding *in vivo* T-cell depletion after reduced-intensity conditioning with alemtuzumab or *ex vivo* T-cell depletion of the graft [19–24]. In a series of 238 paediatric patients, Mynarek *et al.* found that high blood HAdV loads were restricted to patients who received T-cell depletion therapy [19]. Chakrabati *et al.* have shown that the absolute lymphocyte count at 90 to 120 days had a strong impact on HAdV infections in patients who had received alemtuzumab [21]. A recent retrospective analysis of a 2879-patient cohort showed that the frequency of patients with HAdV-disseminated infections was higher when absolute lymphocyte counts were <200/mm^3 [24].

Altogether, these data may suggest that T-cell depletion therapy is not itself a trigger of HAdV replication, but that, once HAdV infection occurs, the T-cell depletion may contribute to the spread of infection, because the T-cell specific immune response does not recover. Assessment of the specific anti-HAdV cellular response could be complementary to HAdV loads for predicting either systemic infection or related disease.

We identified various species in stool and plasma samples. Species C HAdVs were the most frequent in digestive infections, in systemic infections, and in related diseases, as shown in other studies [3,14]. However, species C seems not to be associated with higher pathogenicity, as the ratio between species C and the others species remained the same in HAdV digestive infections, systemic infections, and related disease. The higher frequency of species C than of other species detected in paediatric transplant patients reflects their ability to persist frequently in the digestive tract [25,26]. Species A, which is also associated with digestive infections, was the second most frequent species that we found. As most HAdV infections occurred early after transplantation, the most likely mechanism of digestive infection after transplantation is reactivation rather than new infection. Whether the detection of HAdV in the digestive tract before transplantation would predict post-transplant infection and could help to determine which patients would require HAdV monitoring is an area for further study.

In our study, all patients but one who experienced systemic HAdV infection had the virus detectable in stool specimens. This confirms that the intestinal tract is a common source of virus dissemination [14,26]. When restricting the analysis to the patients with intestinal shedding of HAdV, we found a significant difference in median peak levels of HAdV in stools (>3.5 $\log_{10}$) between patients who experienced HAdV systemic infection and those who did not. GVHD grade >2 and HAdV in stools were the only factors associated with the occurrence of systemic infection in a multivariable Cox model. In addition, we showed that the risk of viraemia remained limited for HAdV loads in stools of <5 $\log_{10}$ copies/mL. In contrast, HAdV viraemia level did not add any prognostic information to that provided by the viral load in stools with regard to the risk of progression to HAdV disease.

Our data corroborate those published by Lion *et al.* They showed an incidence of HAdV viraemia of 73% in patients with a peak of virus levels of >6 $\log_{10}$ copies per gram of stool vs. 0% in patients with HAdV levels below this threshold [14]. Jeulin *et al.* also found that an HAdV DNA level of >5.47 $\log_{10}$ copies per gram of stool preceded HAdV DNA detection in blood [27]. In contrast, Mynarek *et al.* recently reported a higher rate of HAdV blood infections (50.4%) than of digestive infections (43.2%) [19]. The higher incidence of viraemia in that study may be explained by the use of whole blood instead of plasma. The lack of standardization of methods for the quantification of HAdV in stool may also account for some apparent discrepancies.

Altogether, these results thus suggest that quantitative monitoring of HAdV DNA in stool samples could allow the prediction of imminent viraemia in paediatric HSCT patients with risk factors for HAdV systemic infection, and could be used to trigger pre-emptive treatment to control HAdV replication in the digestive tract and to prevent systemic spread of the infection.

Whereas the low concentration of cidofovir in the digestive tract does not enable such a goal to be achieved, CMX001 (brincidofovir), which provides a high tissue concentration, may make this objective more attainable [10,28,29]. The results of a phase 2 study—a randomized, double-blind, placebo-controlled trial evaluating the safety and efficacy of CMX001 for the prevention of HAdV disease in HSCT recipients with asymptomatic HAdV viraemia (ADV HALT Trial, NCT01241344)—are pending. Diarrhoea has been reported as the most frequent adverse event [30]. Because diarrhoea is a sign of either infectious enteritis or GVHD, avoiding or reducing such an adverse effect is desirable. A pre-emptive treatment targeting HAdV digestive replication before the onset of viraemia could reduce the risk of dissemination with shorter treatment duration, thus reducing drug exposure and possibly the risk of digestive adverse events.

Despite the use of antiviral drugs, including brincidofovir, and immunotherapy, the mortality rate of patients with HAdV-related disease was high (61.5%, 8/13), most deaths being related to HAdV infection. We may assume that the intervention was too late for specific antiviral activity to be translated into clinical efficacy. It is of note that four patients received antiviral drugs (cidofovir and brincidofovir) for HAdV digestive infection with rapidly increasing HAdV levels in stools, and none developed HAdV systemic infection.

In conclusion, our study has confirmed paediatric HSCT subpopulations as being at the greatest risk for HAdV disseminated infection that might warrant HAdV molecular screening, and has shown that HAdV DNA levels in stools are predictive of disseminated infection in high-risk patients. On the basis of cumulative data [14,27], we recommend HAdV stool monitoring for paediatric HSCT recipients receiving cord blood transplants or *in vitro* T-cell-depleted transplants, and for patients with severe GVHD. Our results, together with previous reports [14,27], provide a rationale for conducting randomized clinical trials to evaluate the benefit of anti-HAdV pre-emptive treatment interventions based on HAdV DNA levels in stools.

Author contributions

J. LeGoff was the principal investigator and takes primary responsibility for the paper. J.-H. Dalle, A. Baruchel, A. Cuinet, M. Ouachée, K. Yacouben and M. Fahd recruited the patients. L. Feghoul, F. Simon, S. Chevret, V. Guérin-El Khourouj, G. Sterkers, and J. LeGoff organized and supervised the laboratory work for this study. J. Roupret-Serzec summarized antiviral treatments. S. Chevret performed the statistical analysis. L. Feghoul, J. LeGoff, S. Chevret and J.-H. Dalle coordinated the research. J. LeGoff, L. Feghoul, S. Chevret and J.-H. Dalle wrote the paper. G. Sterkers and V. Guérin-El Khourouj reviewed and corrected the first version. All authors reviewed the final version.

Transparency declaration

The authors report no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.03.011.

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